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Characteristics of sodium uptake across the basolateral membrane of oxyntic cells

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Characteristics of sodium uptake across the basolateral membrane of oxyntic cells. To characterize further serosal Na uptake into gastric oxyntic cells under resting conditions, cellular element concentrations were determined in isolated frog (*Rana temporaria*) gastric mucosae using electron microprobe analysis. The epithelia were kept short circuited in Ussing-type chambers, and element analysis was performed on freeze-dried cryosections. After ouabain (10^{-4} M), the [Na] in oxyntic cells increased within 30 to 60 minutes from approximately 25 to 100 mmol/kg wet wt, and [K] decreased similarly (from 100 to 25 mmol/kg wet wt). These changes occurred regardless of whether the basolateral incubation medium contained HCO_3 or N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) as buffers. When, prior to the addition of ouabain, 10^{-3} M amiloride was applied to the serosal side to inhibit the Na-H antiporter, the ouabain-induced increase in cellular [Na] was prevented completely in HEPES-, but not in HCO_3-Ringer. The data are compatible with the notion that Na is taken up by a Na-H antiporter and a Na-HCO_3 symporter. At least under these experimental conditions, these transporters seem to contribute substantially to basolateral Na uptake in oxyntic cells.

Na influx into oxyntic cells is a prerequisite for maintenance of gastric H secretion. Cellular influx of Na and its exchange for K via the basolateral Na-K-ATPase is the initial step in K secretion, which in turn drives the luminal H-K-ATPase. A variety of Na cotransporters (Na-HCO_3 [1], Na-K-2Cl [2], and Na-Cl [3]) and a Na-H-antiporter might contribute to the Na influx in oxyntic cells. To elucidate the contribution of the Na-H-antiporter and the Na-HCO_3-cotransporter to the Na influx in oxyntic cells of the frog gastric mucosa under resting conditions, cellular element concentrations were determined by electron microprobe analysis. The increase in cellular [Na] after blocking Na-K-ATPase by ouabain is assumed to reflect cellular Na influx. N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)-Ringer and amiloride were applied to the serosal side to inhibit the Na-HCO_3-cotransporter and the Na-H-antiporter, respectively.

Key words: N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, HCO_3, ouabain, amiloride, sodium, potassium, chloride, gastric mucosae, antiporter, symporter, transport.

METHODS

Isolated, split frog (*Rana temporaria*) gastric mucosae were kept short circuited in Ussing-type chambers under resting conditions (cimetidine 10^{-4} M). Whereas in all experiments the mucosal side was bathed by HCO_3-Ringer, the serosal Ringer was either buffered by HCO_3 or HEPES. Ouabain (10^{-4} M) and amiloride (10^{-3} M) were applied to the serosal side to inhibit the Na-K-ATPase and the Na-H-antiporter, respectively. After chamber incubation, the epithelia were covered on the mucosal side with a thin layer of albumin standard solution, and freeze-dried cryosections were prepared for electron microprobe analysis. Energy dispersive x-ray spectra were obtained from the cells and the standard layer in a scanning electron microscope (Stereoscan S150; Cambridge Instruments, Cambridge, UK) with a LINK detector (LINK System, High Wighcombe, UK). Cellular element concentrations were quantified by direct comparison of the element-specific x-ray signals of cells with the standard spectra.

RESULTS AND DISCUSSION

Table 1 shows element concentrations in oxyntic cells under control conditions. No systematic differences are present between the element concentrations and dry weight contents obtained from epithelia incubated either with HCO_3- or HEPES-Ringer on the serosal side. [Na] and [Cl] are relatively low, and [K] is high. Under both conditions, the sum of [Na] and [K] was about 120 mmol/kg wet wt.

Figure 1 shows the effects of serosal ouabain and of the successive application of amiloride and ouabain to the serosal side in the presence of either serosal HCO_3- (Fig. 1A) or HEPES-Ringer (Fig. 1B) on [Na] in oxyntic cells. Under both conditions, the [Na] increased after ouabain within 30 minutes from approximately 20 to 100 mmol/kg wet wt. [K] decreased by about the same amount, and [Cl] increased from approximately 35 to 45 mmol/kg wet wt. In the presence of serosal HCO_3-Ringer, the prior addition of

Table 1. Cellular element concentrations and dry-weight contents of resting oxyntic cells of *Rana temporaria* under control conditions

	Na	Mg	P	S	Cl	K	Dry wt
	mmol/kg wet wt						*g/100 g wet wt*
Control (HCO_3)	26.6 ± 5.64	12.3 ± 1.30	146.0 ± 10.50	35.7 ± 2.50	30.8 ± 1.35	97.3 ± 5.76	31.2 ± 6.43
Control HEPES	22.5 ± 3.33	11.6 ± 1.29	143.3 ± 19.5	29.6 ± 2.35	35.2 ± 5.21	98.9 ± 8.67	31.5 ± 5.50

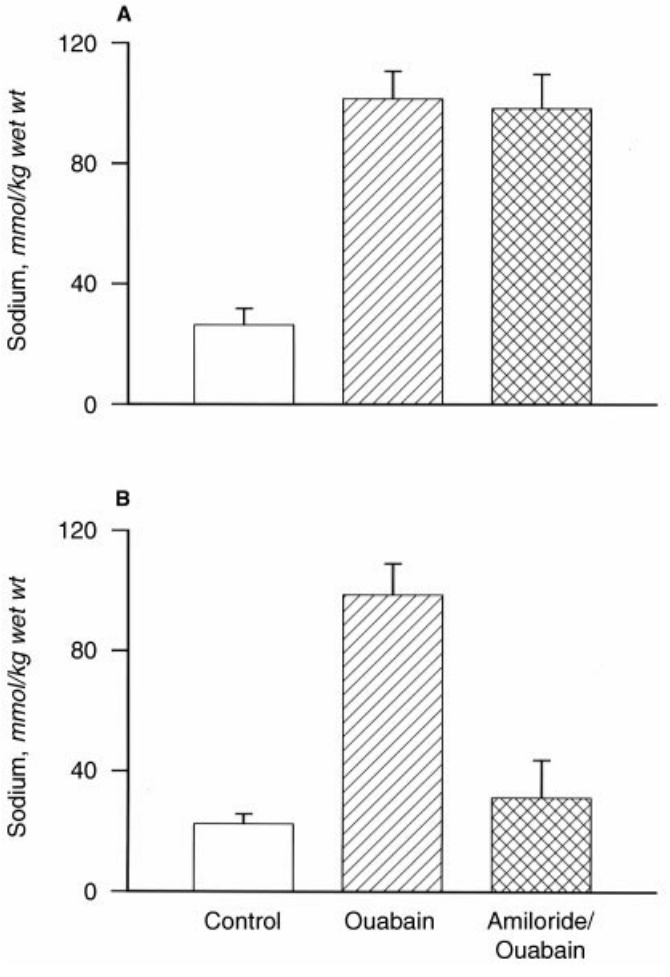

Fig. 1. [Na] in oxyntic cells of frog gastric mucosae in the presence of serosal HCO_3-Ringer (*A*) and HEPES-Ringer (*B*) under control conditions, after ouabain (10^{-4} M, serosal) and after successive application of amiloride (10^{-3} M) and ouabain (10^{-4} M).

amiloride had no influence on the ouabain-induced increase in [Na] (Fig. 1A). In contrast, the ouabain-induced increase in [Na] was almost completely abolished by amiloride in the presence of serosal HEPES-Ringer. In this latter case also, cellular [K] and [Cl] remained normal.

The cellular element concentrations in oxyntic cells under control conditions both with HCO_3- and HEPES-Ringer on the serosal side were similar to those measured by electron microprobe analysis in other amphibian epithelia. After ouabain, regardless of the buffer used on the serosal side, almost complete exchange of cellular K with extracellular Na resulted in an increase of cellular [Na] to 100 mmol/kg wet wt. Whereas rat gastric gland cells behave similarly [4], the Na activity determined by a fluorescent dye in rabbit parietal cells increased to only 45 mM [5]. Whether this discrepancy is due to species differences or can be explained by the different incubation solutions or the different measuring techniques cannot be answered. Because amiloride blocks the Na-H-exchanger in the serosal membrane of H-secreting gastric cells, these results obtained after the successive application of amiloride and ouabain can be interpreted as follows. The finding that the ouabain-induced Na increase could be blocked by amiloride in the presence of serosal HEPES-, but not HCO_3-Ringer, agrees with the view that Na influx into resting oxyntic cells is accomplished by a Na-H-exchanger [5] and a Na-HCO_3-cotransporter [1] in the basolateral membrane. As already demonstrated for rat gastric gland cells [4] also oxyntic cells of the frog stomach seem to take up Na only from the serosal side. These experiments yield no evidence that Na-K-2Cl [2] or Na-Cl cotransport [3] is involved in cellular Na influx in the resting state. Because in those cases in which ouabain had an effect on the cellular element composition (serosal HCO_3-Ringer with or without amiloride and serosal HEPES-Ringer) the Na-K exchange was complete within the ouabain incubation time of 30 minutes, the contribution of either Na-H exchange or Na-HCO_3 cotransport to Na influx cannot be assessed precisely. However, the Na influx thought to be accomplished by the Na-HCO_3-cotransporter led to a [Na] increase of at least 2 to 3 mM/min. This is similar to that found in rabbit parietal cells for the Na-H exchanger [5]. Assuming this to be so, the Na-HCO_3-cotransporter may be important in the regulation of the intracellular pH in frog oxyntic cells. That such a role for Na-HCO_3 cotransport is not found in isolated rabbit parietal cells [6] might be explained by differences in the H-secreting cells of frog and rabbit stomach or by the different preparation techniques used.

In summary, the data provide evidence that Na influx into resting frog oxyntic cells is accomplished by an amiloride-sensitive Na-H exchanger and a Na-HCO_3 cotransporter.

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